AD______________

AWARD NUMBER: W81XWH-12-1-0205

TITLE: Novel Therapeutic Target for the Treatment of Lupus

PRINCIPAL INVESTIGATOR: Lisa Laury-Kleintop, PhD

RECIPIENT: Lankenau Institute for Medical Research, Wynnewood, PA 19096

REPORT DATE: December 2013

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release; Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE
December 2013

2. REPORT TYPE
Annual

3. DATES COVERED
01 July 2013 – 30 November 2013

4. TITLE AND SUBTITLE

Novel Therapeutic Target for the Treatment of Lupus

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-12-1-0205

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Lisa Laury-Kleintop, Ph.D.

E-Mail: KleintopL@mlhs.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Lankenau Institute for Medical Research

Wynnewood, PA 19096

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Many therapeutic strategies for SLE focus on the central role that autoantibody-producing B cells play in the pathology of this disorder. One general immunotherapeutic theme employs monoclonal antibodies (mAb) to interfere with, and/or deplete, B cells to ultimately reduce disease causing autoantibody levels. However, current strategies are inherently limited because they are not specific for the disease state. Thus, treatments that can specifically block autoantibody production without compromising B cell function are needed. In our application we presented preliminary evidence in an *in vivo* model of a related autoimmune disease (rheumatoid arthritis) that showed antibodies to RhoB, a small GTPase blocked autoantibody secretion without affecting the overall B cell repertoire. This data led us to propose the purpose of this study, to evaluate the ability of anti-RhoB antibodies to reduce levels of pathologic autoantibodies, ultimately attenuating the severity of symptoms in the MRL-lpr murine model of SLE. We have not yet completed testing the anti-RhoB therapy in the SLE model and subsequently received approval of an extension without funds for the award period. Thus far, our data suggests that dosing with the anti-RhoB antibodies produces a trend towards a decrease in autoantibody titers and proteinuria; however, we are currently performing another treatment experiment that we hope will enable us to achieve statistical significance and have a better understanding of the effects resulting from targeting RhoB.

15. SUBJECT TERMS
RhoB, animal model, antibody secretion, antibody therapy, Systemic lupus erythematosus, autoantibodies.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	6	

Table of Contents

1. INTRODUCTION:

Systemic lupus erythematosus (SLE) affects approximately 300,000 to over a million individuals in the United States with a female gender bias of 90%. Many therapeutic strategies for SLE focus on the central role that autoantibody-producing B cells play in the pathology of this disorder. One general immunotherapeutic theme employs monoclonal antibodies (mAb) to interfere with and/or deplete B cells to ultimately reduce disease causing autoantibody levels. However, current strategies are inherently limited because they are not specific for the disease state. Thus, treatments that can specifically block autoantibody production without compromising B cell function remain lacking. Our work aims to address this therapeutic gap. The objective of our study is to evaluate the ability of anti-RhoB peptide antibodies to reduce levels of pathologic autoantibodies, ultimately attenuating the severity of symptoms in the MRL-lpr murine model of SLE. Our research strategy includes the determination of autoantibody levels over the course of disease progression in animals treated with therapeutic and control antibodies. Additionally, autoantibody levels will be compared to renal pathology to correlate autoantibody levels with attenuation of disease symptoms. This study will lay the groundwork for developing an innovative therapeutic strategy to improve the care of SLE patients, address important and timely scientific questions, as well as, focus on a major unmet medical need.

2. KEYWORDS:

RhoB, animal model, antibody secretion, antibody therapy, Systemic lupus erythematosus, autoantibodies.

3. OVERALL PROJECT SUMMARY:

Objective to complete in the award period of 18 months.

Evaluate the ability of anti-RhoB antibodies to attenuate the severity of symptom in the MRL/MpJ-Faslpr (abbrev. MRL-lpr) animal model of SLE.

Task I. Prepare anti-RhoB antibody. *Months 1-3*
Task II. Obtain approval for animal study. *Months 1-3*
During the first 3 months of the award period the first two tasks listed in the SOW were completed. During this time we refined the methodology and began purification of the RhoB antibodies (7F7 and 9G5), as well as, completed the approval process for our animal studies. Unfortunately, during this time it was determined that we had a mite outbreak in the animal facility and this outbreak has complicated the completion of the remaining tasks.
Task III. Obtain MRL/MpJ-Faslpr female mice from approved vendor. *Month 3*
Task IV. Administer anti-RhoB antibody to MRL-*lpr* mice and monitor for the development of autoantibodies and proteinuria. Mice will be dosed regularly during the course of the experiment, approximately 6 months. We anticipate repeating the experiment to reach statistical significance. *Months 3-15*
Task V. Perform ELISA analyses to evaluate serum autoantibody levels. *Months 3-15*
Task VI. Final serum autoantibody levels will be determined, as well as the levels of cells actively secreting anti-chromatin antibodies by ELISpot from isolated spleens. Renal function will be determined by monitoring for proteinuria. Renal pathology will be scored for nephritis using histological kidney sections. *Months 10-16*
Task VII. Statistically analyze the data. *Months10-18*

We are currently working to complete tasks 3 through 7.

<u>Text included in the Progress Report submitted July 2013</u>. Because of the detection of mites in the animal facility we did not import mice until the treatment for the outbreak was completed. All mice in the facility were treated topically with a 5% moxadectin solution (August 2012). We waited to import

mice and begin treatment until testing results indicated the mite outbreak was cleared (October 2012). However, approximately 3 weeks after we had imported and begun treating the first set of MRL-lpr mice with our targeted therapy, mites were again found in the facility. With this second outbreak all animals in the facility were fed ivermectin-containing food, and in our study, we continued to treat the MRL-lpr mice with our RhoB-targeted therapy. Analysis of the autoantibody levels from this group of mice did not show differences between the groups. However, we are concerned about the significance of the result from this first set of animals because ivermectin treatment has been shown to affect immune responses [1-3]. Additionally, in two other mouse models of autoimmune disease that received the ivermectin containing food, changes were observed in altered disease development and antibody levels as compared to studies done prior to the detection of mites and subsequent ivermectin treatment.

In February 2013, we imported a second group of MRL-lpr mice after ivermectin treatment ended and sentinel screening was clear. The mice were divided into 3 treatment groups that received anti-RhoB antibody 7F7 or 9G5, or mouse IgG as a control. Dosing began at 4 weeks of age and continued one dose per week until the end of the experiment. We assessed the development of serum autoantibody titers and proteinuria. The data presented in Figure 1, panel A suggest a trend towards a decrease in titers for anti-double stranded DNA antibodies in the groups treated with the anti-RhoB antibodies compared to control IgG. Additionally, the data shown in Figure 1, panel B suggests a trending decrease in proteinuria with treatment of the anti-RhoB antibodies. None of these differences reached statistical significance. Kidney tissue from these mice was isolated and histological samples will be scored to assess renal pathology. The data presented in Figure 1 suggests that anti-RhoB antibodies may affect autoantibody titer and proteinuria in MRL-lpr mice; however, additional numbers are needed to achieve statistical significance.

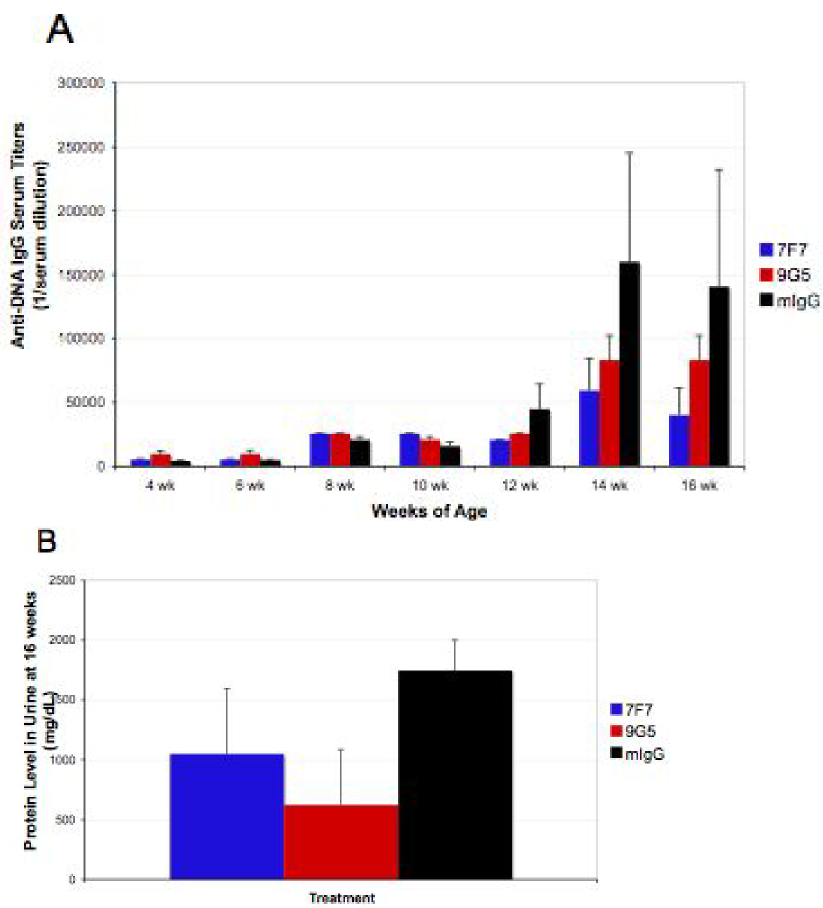

Figure 1: Autoantibody and proteinuria levels from MRL-lpr mice treated with anti-RhoB antibodies 7F7 or 9G5. Panel A: ELISA results for serum antibody titers to double-stranded DNA. Serum titers were determined at indicated ages during the treatment period. Anti-RhoB antibodies 7F7 and 9G5 were compared to control mouse IgG. Panel B: Proteinuria was determined at 16 weeks of age prior to euthanasia. N=4-5 mice per group.

During the remaining period of the award we will repeat our analysis using additional mice. We are in the process of adding animal numbers to our IACUC and DOD animal protocols. Once appropriate approvals have been received we will continue with our study.

<u>We have two items to report since the submission of the Progress Report in July 2013.</u>
First, we have received approval of our amended animal protocol from both our IACUC and the DOD. Once approved, we purchased mice to continue our study. The mice were divided into a control group that is receiving control IgG or a treatment group receiving anti-RhoB antibody 7F7. We chose to focus our attention on the effects of 7F7 because of new data regarding the antigen affinities of 7F7 and 9G5. Using recombinant proteins we characterized the cross-reactivity of the antibodies for other members of the small GTPase family. We found that 7F7 only recognized RhoB in elisa assays, while 9G5 recognized RhoB, RhoA and cdc42. Because 7F7 appears specific for RhoB, we plan to determine its therapeutic potential. It is still early in the treatment period and over the coming months we will perform assays to monitor autoantibody levels.

Second, we had the kidney tissue from second cohort of mice (February 2013) scored to assess renal pathology. The results suggested that mice in the 9G5 treated group had a reduced degree of renal involvement exhibited by attenuated glomerular cellularity and basement membrane thickening. In light of our affinity data that 9G5 showed cross-reactivity with RhoA and cdc42, it may be that one or both of these other small GTPases play a role in the development of renal disease in this syndrome. We will have kidney tissue scored for renal disease from our current groups once treatment has been completed.

4. KEY RESEARCH ACCOMPLISHMENTS:
There are no research accomplishments thus far from the study.

5. CONCLUSIONS:
At present we are not able to conclude that our therapy can affect autoantibody levels in the MRL-lpr model of SLE. We will continue to work to meet the objective of our study, to evaluate the ability of anti-RhoB peptide antibodies to reduce levels of pathologic autoantibodies, which we believe will attenuate the severity of symptoms in this animal model. Despite the setback of fur mites in the animal facility, we plan to meet our goal and obtain preliminary data necessary to advance an innovative therapeutic strategy into the clinic for treatment of SLE patients.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS: None.

7. INVENTIONS, PATENTS AND LICENSES: None.

8. REPORTABLE OUTCOMES: There are no reportable outcomes thus far from the study.

9. OTHER ACHIEVEMENTS: None.

10. REFERENCES:

1. Blakley, B.R. and C.G. Rousseaux, *Effect of ivermectin on the immune response in mice.* Am J Vet Res, 1991. **52**(4): p. 593-5.
2. Yan, S., X. Ci, N. Chen, C. Chen, X. Li, X. Chu, J. Li, and X. Deng, *Anti-inflammatory effects of ivermectin in mouse model of allergic asthma.* Inflamm Res, 2011. **60**(6): p. 589-96.
3. Roble, G.S., W. Boteler, E. Riedel, and N.S. Lipman, *Total IgE as a serodiagnostic marker to aid murine fur mite detection.* J Am Assoc Lab Anim Sci, 2012. **51**(2): p. 199-208.

11. APPENDICES: None.